Diversity of Avian Spermatozoa Ultrastructure with Emphasis on the Members of the Order Passeriformes

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ABSTRACT

The spermatozoa of passerine birds have ultrastructural features which are common to the sperm of members of the other orders of the class Aves. The sperm are filiform, the acrosome is conical in shape, and the nucleus is about the same diameter as the acrosome and midpiece. The helical shape of the passerine sperm, the presence of a helical membrane extending laterally from the acrosome, the 9 peripheral dense fibres (accessory fibres) which extend from a juxtanuclear body along the doublets of the axoneme, and the presence of a single elongated helical mitochondrion are synapomorphies of the passerines. Since avian spermatozoa exhibit considerable variation in gross morphology and in ultrastructure, these variations may be useful indicators of phylogenetic relationships. It is necessary to have details of the fine structure in order to develop a tree of these taxonomic relationships. The Class Aves is divided into a number of orders based on a number of features including morphology, and behaviour. The order Passeriformes contains 5712 (59%) of the 9672 species of recent birds. Most of the passerine birds are small land dwelling birds that tend to exhibit similar morphological features thus making their subdivision into categories below suborders difficult. This study compares some of the ultrastructural differences in sperm of selected members of the order Passeriformes.

RÉSUMÉ

Diversité de l'ultrastructure des spermatozoïdes d'Oiseaux, en particulier des Passeriformes

Les spermatozoïdes des Oiseaux Passeriformes ont des caractéristiques ultrastructurales qui sont communes aux spermatozoïdes des membres des autres ordres de la classe Aves. Les spermatozoïdes sont filiformes, l'acrosome est conique et le noyau est approximativement du même diamètre que l'acrosome et la pièce intermédiaire. La forme hélicoïdale du spermatozoïde, la présence d'une membrane hélicoïdale qui s'étend latéralement à partir de l'acrosome, les 9 fibres denses périphériques (fibres accessoires) qui partent d'un corps juxtanucléaire le long des doublets de l'axonème, et la présence d'une mitochondrie unique, allongée et hélicoïdale sont des synapomorphies des Passeriformes. Comme les spermatozoïdes des Oiseaux montrent des variations considérables de morphologie générale et d'ultrastructure, ces variations peuvent être d'utiles indicateurs des relations phylogéniques. Il est nécessaire de connaître les détails de l'ultrastructure pour créer un arbre des relations taxonomiques. La classe Aves est divisée en plusieurs ordres basés sur de nombreux caractères incluant la morphologie et le comportement. L'ordre des Passeriformes contient 5712 (59%) des 9672 espèces d'oiseaux récents. La plupart des Passeriformes sont de petits oiseaux terrestres qui tendent à montrer des caractéristiques morphologiques similaires, ce qui rend difficile leur classification au niveau infraordinal. Cette étude compare certaines des différences ultrastructurales des spermatozoïdes parmi des membres sélectionnés de l'ordre Passeriformes.

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About 200 million years ago, Pangaea was breaking up to form Laurasia and Gondwanaland. The dominant terrestrial life forms were, it is believed, the dinosaurs. The radiation of the birds probably began about this time. A number of traits are used to construct the taxonomic and phylogenetic relationships between the various members of the class Aves, but it has been difficult to resolve the taxonomic relationships. According to SIBLEY & AHLQUIST [32] most passerines are small land dwelling birds that feed primarily on insects, seeds, fruit or nectar. The morphology of the syrinx is used to separate the members of the suborders Tyranni and the Passeri. As birds evolved many have undergone convergent evolution to allow distantly related groups to cope with similar environmental demands while others have undergone divergent evolution so that related groups may look very different. Homologies or similarities due to common ancestor must be recognized to demonstrate taxonomic relationships. It has been known since the works of BALLOWITZ [7] & RETZIUS [29, 30] that morphological differences are exhibited by the spermatozoa of various members of the Class Aves. Each noted especially the differences in the sperm of passerine and non-passerine sperm. More recent studies of spermiotaxonomy and spermiocladistics [19] have been useful in resolving taxonomic relationships in other groups of organisms.

Since avian sperm exhibit considerably morphological variability it will be useful to examine the ultrastructure of sperm as it relates to the phylogeny. A number of studies have given descriptions of the ultrastructure of spermatozoa of representatives of orders of non-passerine birds. The literature contains a few reports of ultrastructure of passerine spermatids and sperm in sections of testis. There is currently very little information on the ultrastructure of mature passerine sperm. The Order Passeriformes contains approximately 59% of all of the species of living birds, and the order is divided into two suborders and generally into 45 families [31, 32]. It is of interest to examine and compare the ultrastructure of the mature sperm of some representative members. Reconstructing the phylogenetic history of members of the order is a major challenge. In this paper I will follow the classification of SIBLEY *et al.* [32] in which the order Passeriformes is divided into the Suborders Tyranni (= Suboscines; Oligomyodi) and Passeri (= Oscines; Polymyodi). The suborder Passeri includes the parvorders Passerida (which includes three superfamilies and 21 families. The suborder Corvida includes three superfamilies and 15 families. MCFARLANE [23, 24] noted that the avian sperm exhibit considerable species variation in both size and morphology, thus it is here considered that the structure of sperm should yield phylogenetic information. ASA & PHILLIPS [3] described some differences between the oscines and sub-oscines.

The sperms of non-passerine birds are generally nearly linear in the head region while the sperms of passerine birds are helically coiled. Tripepi and Perotta [35] examined oscine spermiogenesis and divided the oscines (Passeri) into two types based on the presence of a granular body and the ratio of length of the nucleus to the length of the acrosome. The present work will describe the morphological variation of the sperm of various passerine birds and will compare these sperm with those of some non-passerine birds.

MATERIALS AND METHODS

Sperm specimen were obtained from various species of birds of the Order Passeriformes. All birds were from the suborder Passeri, and represented five different families. Species included *Agelaius phoeniceus, Molothrus ater, Quiscalus quiscula, Sturnus vulgaris, Cardinalis cardinalis,* and *Passer domesticus.* Male birds were obtained during the breeding season, euthanased, and sections of the distal portion of the vas deferens and/or the seminal glomeruli were removed, fixed in 2% glutaraldehyde in phosphate buffer, pH 7.2, post fixed in 1% osmium. For TEM specimen were dehydrated in acetone, embedded, sectioned, stained with uranyl acetate and lead citrate, and examined with a Philips 300. For SEM the fixed specimen were dehydrated, critical point dried sputter coated and examined with an AMRAY 1200.

RESULTS

The sperm of each species is characterized by being long and cylindrical in overall morphology, with the diameter of the acrosome, nucleus and midpiece being nearly the same. Table 1 gives the various features and measurements of the sperm.

The sperm possess a helical shape, especially in the region of the acrosome and nucleus, generally with the helical coiling ending at the neck. The degree of coiling and the length between turns of the helix is variable in sperm of different species. The sperm also possess a helical membrane starting near the anterior tip of the acrosome and extending over the length of the acrosome to the anterior end of the nucleus. The helical appearance of the midpiece and tail generally is due to the coiling of an elongated granular mass followed posteriorly by the mitochondrial sheath surrounding the neck and midpiece, and extending posteriorly to the endpiece.

The acrosome at the anterior end of the head is characterized by having an electron dense core surrounded by a more electron lucent region. Considerable differences are noted in the length of the acrosome in relation to the length of the nucleus. In some species there is an anterior nuclear fossa of variable depth to 1.1 µm. A perforatorium was not observed in any of the passerine sperm. The neck of the sperm do not appear to have typical distinct centrioles. The flagella are composed of an axoneme having a 9+2 arrangement, surrounded by 9 dense accessory fibres extending from the neck to the posterior end of the principal piece. A posterior nuclear fossa does not appear to occur in the sperm of these passerines studied. Morphology of passerine sperm differs greatly from that of the sperm of non-passerine birds.

TABLE 1. — The sperm of passerine birds exhibit considerable variation in size and morphology. Measurements from scanning and transmission electron micrographs are presented.

Family	Passeridae	Tyrannidae	Hirundinidae	Paridae	Vireonidae	Turidae	Thraupidae
Taxon	*Passer domesticus*	*Tyrannus verticalis*	*Tachycineta thalassina*	*Parus bicolor*	*Vireo olivaceus*	*Turdus migratorius*	*Piranga rubra*
Common name	House Sparrow	Western Kingbird	Violet-green Swallow	Tufted Titmouse	Red-eyed vireo	Robin	Summer Tananger
Sperm length	77 µm	55 µm	285 µm	90 µm	80 µm	70 µm	170 µm
Acrosome length	6-7 µm	2.5 µm	13.5 µm	7.1 µm	8.0 µm	6.7 µm	12 µm
Acrosomal Core	Yes		Dense core				
Nuclear Length	6 µm	14 µm	4.5 µm	5.8 µm	5.5 µm	2.5 µm	3 µm
Granular material at midpiece	yes						
Midpiece Length	55 µm	4 µm	granular material at midpiece	50 µm	10 µm	46 µm	146 µm
Helical Shape	yes	yes	yes	yes	yes	yes	yes
Helical Membrane	Yes -only on acrosome		Yes-acrosome only	acrosome only	acrosome only	acrosome only	acrosome only
Perforatorium	No		no				
Anterior Nuclear Fossa	Yes 0.7-1.08 µm		yes 0.7 µm	no	no	no	no
Posterior Nuclear Fossa	no	yes shallow	no	slight	no	no	no
Centrioles	Distal only		distal only	distal			
Accessory Fibres	yes-attached to mitochondrion	yes	yes - attached to axomeme at anterior end	yes	yes	yes	
Mitochondria	1 elongated helical - posterior to the granular material		1 or 2, long one is helical		1 helical	Helical large	
Annulus	not observed		no				
Reference	Present study	[24]	[24]	[24]	[24]	[24]	[24]

TABLE 1, continued

Family	Emberizidae	Sturnidae	Icteridae	Icteridae	Icteridae	Tyrannidae
Taxon	*Cardinalis cardinalis*	*Sturnus vulgaris*	*Molothrus ater*	*Agelaius phoeniceus*	*Quiscaous quiscula*	*Myiarchus crinitus*
Common Name	Cardinal	Starling	Brown-headed cowbird	Red-winged blackbird	common grackle	Great Crested Flycatcher
Sperm length	76 µm	50 µm	62 µm	110 µm	48 µm	50 µm
Acrosome length	6.6 µm	2.5 µm	6.0 µm	8.0 µm	7.0 µm	2.5µm
Acrosomal Core						Uniform throughout
Nuclear Length	4.0 µm	3.0 µm	3.0 µm	5.0 µm	4.0 µm	18.5 µm
Granular body at midpiece		yes	yes		yes	
Midpiece Length	35. µm		24. µm			3.3µm
Helical Shape	yes	yes	yes		yes	Acrosome, nucleus & midpiece
Helical Membrane	yes	yes	yes		yes	Acrosome only reduced size
Perforatorium						No
Anterior Nuclear Fossa			shallow		shallow	
Posterior Nuclear Fossa			yes		yes	shallow
Centrioles					distal	–
Accessory Fibers			yes		yes	Yes
Mitochondria			spiral			Helical One revolution
Annulus						none
Reference	Present study	Present study	Present study	Present study	Present study	[24]

DISCUSSION

General

The overall length of the sperm of passerine birds is highly variable, the extremes noted in those studied are 48 µm for the *Quiscalus quiscalus* sperm to 285 µm for the *Tachycineta thalassina* [24] sperm. The overall appearance ranges from having a helical structure only in the acrosomal region to having a helical acrosome, nucleus and a helical midpiece.

The acrosome of all of the passerine birds in this study is a solid, helical apical body with a helical membrane generally running from the acrosome tip to the posterior end of the acrosome. The helical membrane is a lateral extension of the acrosome and forms a left handed helix. The size of this helical membrane is highly variable. Helical membranes or helically elongate nuclei are also recorded in a variety of invertebrates such as the cephalopod and gastropod molluscs [14], chilopods [18] and leeches [12]. The overall length of the acrosome is highly variable ranging from 2.5 µm in the sperm of *Sturnus vulgarus* to a long structure that dominates the head and is several times as long as the nucleus in the sperm of *M. crintus*. The acrosome generally appears to be covered by an outer acrosomal membrane which lies just beneath the plasma membrane. The acrosomal matrix generally has two different components that can be recognized by their density in the electron beam. The acrosomes have a dense inner core and a less dense outer cortex, there is not a membrane or a space separating these regions. This is in contrast to the acrosome of the domestic fowl sperm in which there is a dense outer cortex and a less dense inner core. The less dense outer material is continuous with the space in the helical membrane. MCFARLANE [24] described membrane-limited vacuoles in the contents of the acrosome of *C. carolinus*, Vacuoles were not observed in any sperm in the present study.

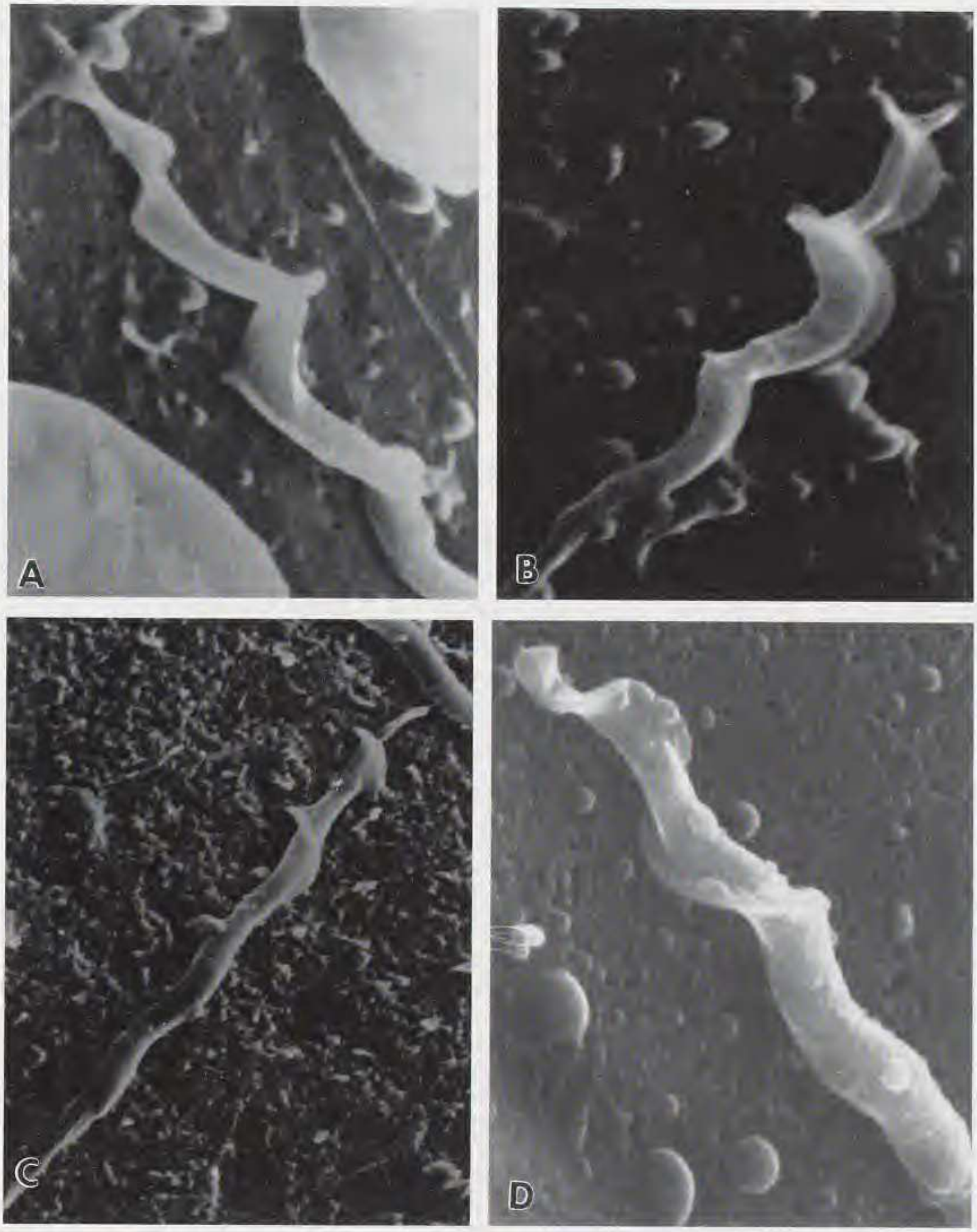

FIG. 1. — Sperm of four species of passerine birds were fixed on filters, dehydrated, critical point dried, coated with gold-palladium, and examined with a scanning electron microscope. The figure shows the acrosomal region. Note that some sperm, have a nearly linear acrosomal axis while others have a spiral shaped axis; all have a helical membrane. **a:** *Quiscalus quiscula*, Common grackle. x 8 200. **b:** *Agelaius phoeniceus*, Red-winged Blackbird. x 10 500. **c:** *Passer domesticus*, House Sparrow. x 11 200. **d:** *Cardinalis cardinalis*, cardinal x 11 200.

Nuclei

The nuclei of the sperm of passerine birds tend to be continuous with the helical shape of the acrosome and midpiece; the wavelength of the nuclear axis is highly variable and is probably characteristic of the family [22, 24]. As an example, the nucleus of the sperm of *Passer domesticus* makes one complete helical revolution over its total length. The nucleus is enclosed in a nuclear envelope which is associated closely with the plasma membrane. The chromatin condenses during spermiogenesis. In the mature sperm the nuclear material is rarely homogeneous, usually containing a number of small irregular cavities, randomly distributed, which appear to result from incomplete condensation of the chromatin.

In passerine sperm the nucleus may show a distinct helical shape. The nucleus of most non-passerine sperm is nearly straight although it may demonstrate a slight curvature, as in *Gallus domesticus*, but it is not helically coiled.

The sperm of passerine birds may have a small anterior nuclear fossa, a cone shaped depression into which the posterior extent of the acrosome extends. There are no endonuclear canals present in passerine bird sperm.

Midpiece

The centriolar region has been called the juxtanuclear apparatus by SOTELO & TRUJILLO-CENÓZ [33].

The components in the neck of the passerine sperm are not easily distinguished. SOTELO & TRUJILLO-CENÓZ [33] gave a partial description of the ultrastructure during spermiogenesis for the house sparrow. There are some modifications in the neck as the spermatids mature. Passerine sperm generally appear to lack a proximal centriole. They have a juxtanuclear body which is sometimes referred to as a modified distal centriole. This juxtanuclear body in the mature sparrow sperm appears as a striated, inverted conical structure when viewed in longitudinal sections. In cross sections the juxtanuclear body appears as irregular fibrils attached to the inner margins of the accessory fibres. The axonemal axis does not extend into the juxtanuclear body.

The axoneme is composed of the classical 9+2 arrangement of microtubules, originating a short distance posterior to the juxtanuclear body. The axoneme is surrounded by nine outer accessory fibres. The presence of these accessory fibres in passerine sperm is a feature that distinguishes them from the sperm of non-passerine birds. The accessory fibres have a tear drop shape in cross section with the point toward the associated pair of axonemal microtubules. The anterior ends of the accessory fibres form a connecting piece at the base of the nucleus, and surround the juxtanuclear body [10, 21, 24, 25]. Nine extremely short accessory fibres have been described in the sperm of certain primitive birds, located adjacent to the axoneme in the region of the annulus. These primitive bird sperm also contain a dense fibrous sheath which is located immediately posterior to the annulus and which surrounds the accessory fibres [5]. Very short accessory fibres have also been described in sperm of Galliformes [34] and the mallard duck [17]. These fibres are greatly reduced in both diameter and length when compared with the accessory fibres of passerine sperm. Outer accessory fibres are also found in the sperm of mammals [8, 26], of Tuatara [16], and in molluscan sperm [15]. In passerine birds the fibres are all of similar size and shape whereas the fibres in molluscs are of various sizes in the same sperm and in reptiles the doublets at position 3 and 8 are enlarged [16]. These outer accessory fibres in passerine sperm appear to be relatively rigid. When living passerine sperm are observed in a physiological saline or in water they appear to maintain a rigid straight morphology. The movement appears to be due to the circular motion of the rigid spermatozoon.

A spiral granular mass is present in the anterior region of the midpiece of some passerine sperm [17, 21, 24, 33]. In sperm of some species this granular mass forms a spiral mass located anterior to the nitochondrion, as observed in *Passer domesticus*, with the anterior end of the mitochondrion being a few micrometres caudal to the nucleus. In contrast, in other species the anterior end of the mitochondrion is at the neck and the granular mass is located around the mitochondrion and the axoneme for a short distance.

All passerine sperm appear to have a single very elongated mitochondrion that may extend almost to the end of the midpiece. In the mature sparrow sperm the mitochondrion extends nearly to the end of the tail and the principal piece is nearly non-existent. The mitochondrion appears to attach to the accessory fibres associated with the axoneme at each end. The mitochondrion shows a gradual decrease in its diameter near the posterior end of the flagellum. The cristae in the mitochondrion are tubular in form and show a random arrangement when sectioned material is viewed.

An annulus was not observed in any of the passerine sperm studied. An annulus is present during spermiogenesis in the grackle [21] but it has not been observed in the mature sperm. The sperm of psittaciforms also lack an annulus [20] but they show a clearly demarcated midpiece-tail junction. In the passerine sperm the posterior end of the midpiece is demarcated by the end of the single helical mitochondrion.

If the sperm of passerine birds are compared with those of the non-passerine birds the notable differences include the presence of the helical membrane in the passerines (representatives in two orders of non-passerine birds do possess helical sperm, the Charadriiformes and Procellariformes, but it is believed that this membrane arose independently of the membrane in passerine sperm), the helical shape of the nucleus, the lack of a perforatorium , the lack of the endonuclear canals, the lack of distinct centrioles, the presence of the nine accessory fibres connected to a juxtanuclear apparatus and the lack of an annulus.

Passerine sperm differ significantly from the sperm of the non-passerines but an ultrastructural examination of spermatozoa of many more passerine species is necessary to reveal phylogenetic relationships between the passerine birds.

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Proteins